Transmembrane potential changes and ventricular fibrillation during repetitive myocardial ischaemia in the dog[1]

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SUMMARY Electrophysiological changes occurring soon after experimental coronary occlusion have been examined in 13 dogs by combined recording of epicardial action potential, endocardial-epicardial conduction time, and epicardial ST segment elevation during successive 5-minute occlusions of the left anterior descending coronary artery. After initial coronary occlusion, action potential duration shortened from 182 ± 9 ms to 113 ± 9 ms at 2 minutes and conduction time was prolonged from 24 ± 1 ms to 71 ± 17 ms associated with progressive ST segment elevation. These changes were followed by the appearance of potentials of 'slow response' type morphology, alternans of action potential duration and amplitude, alternans of epicardial and endocardial ST segment elevation, and intermittent conduction block. Ventricular fibrillation was preceded by action potential alternans in 95 per cent and by conduction block in 65 per cent of instances and occurred at a time of maximum conduction delay. These data strongly support the concept of re-entry in the pathogenesis of early ventricular fibrillation.

Changes in action potential duration and conduction time were significantly greater during the first occlusion compared with subsequent occlusion periods. There was no significant difference however between data taken from occlusions 2, 3, 4, or 5. This questions the validity of experimental studies of myocardial ischaemia based on comparisons of sequential coronary occlusions using the first as the control.

The high incidence of arrhythmias and sudden death occurring in the first 2 hours of ischaemia in man can be related to the early phase of malignant ventricular arrhythmias that occurs soon after coronary occlusion in experimental animals (Harris, 1950; Oliver, 1976). Understanding of the pathogenesis of such arrhythmias in experimental animals has been largely derived from *in vitro* measurement of electrophysiological variables (Cranefield *et al.*, 1972; Wit *et al.*, 1974) or from intracellular recordings in the isolated perfused heart (Kardesch *et al.*, 1958; Downar *et al.*, 1977b). Pronounced slowing of conduction and heterogeneity of refractoriness have been shown in the ischaemic myocardium consistent with the initiation of arrhythmias by re-entrant excitation (Han, 1969; Cox *et al.*, 1973; Scherlag *et al.*, 1974; Russell and Oliver, 1978).

Little is known however of the relation between transmembrane potential changes and arrhythmias in acute ischaemia. Assessment of this relation is most relevant when examined in the in situ heart and is possible by using the floating microelectrode technique (Woodbury and Brady, 1956; Prinzmetal *et al.*, 1962; Czarnecka *et al.*, 1973). We have recently described a technique for combined intracellular and extracellular recordings of action potential in vivo during acute myocardial ischaemia suited to the assessment of arrhythmogenesis (Russell *et al.*, 1977), and using this combined technique, we report in the present study (a) the relations between intracellular potential changes, endo-epicardial ST segment elevation, and the onset of ventricular fibrillation after coronary artery occlusion, and (b) the reproducibility of these measurements after successive brief coronary artery occlusions in the anaesthetised dog.

Methods

Thirteen mongrel dogs (weight 11 to 15 kg) were

anaesthetised with sodium pentobarbitone (30 mg/kg intravenously followed by a maintenance infusion of 3 mg/kg per h controlled by a Harvard infusion pump; additional supplements of 3 mg/kg were occasionally required). Sodium chloride 0·9 per cent was infused at 50 ml/h throughout the experiment through a jugular or femoral vein. After intubation with a cuffed endotracheal tube and controlled ventilation with room air, a lateral thoracotomy was performed through the fifth left intercostal space. The heart was suspended in a pericardial cradle and the left anterior descending coronary artery was dissected free 2 to 3 cm from its origin and above its major diagonal branches. Arterial pressure was measured through a femoral arterial cannula using a Statham P23Db strain gauge transducer and displayed on a Devices chart recorder.

Bipolar pacing electrodes were sutured to the left atrial appendage and the heart was paced at 175 beats/min. In 5 dogs, atrial pacing was combined with right vagal stimulation (5 to 10 mA constant current pulse of 4 ms duration at 25 Hz).

Epicardial electrograms were recorded from 5 small disc electrodes (diameter 5 mm) sutured with fine silk to the epicardium within the potentially ischaemic zone of myocardium served by the exposed coronary artery; the electrodes were placed at least 1 cm distant from the probable border of the potentially ischaemic zone and recordings were only obtained after loss of injury potential. A mean signal was derived from an electronic averaging box which also allowed monitoring of any individual lead if required. The endocardial electrogram was recorded within the centre of the potentially ischaemic zone with a plunge electrode of fine insulated copper wire (diameter 0·11 mm) approximately subjacent to the site of epicardial intracellular potential recording.

The cardiac action potential was recorded using our previously reported modification of the floating electrode technique (Russell *et al.*, 1977). Optimal recordings are obtained with thick-wall glass electrodes (2·5 mm external diameter; 1 mm internal diameter) rather than conventional electrodes (1 or 2 mm external diameter). The electrodes were pulled vertically with a slow early phase and a rapid late phase, and then filled with 3M KCl by boiling under reduced atmospheric pressure. Prepared in this way, an acceptable electrode with a short distal tip and an electrical resistance of 10-15 MΩ was obtained. Two floating electrodes were suspended from micro-manipulators and positioned within 1 mm of each other in the centre of the potentially ischaemic area of myocardium. One electrode was impaled to secure an intracellular record, and the other was used as an extracellular indifferent electrode. In some experiments the tip of a fine insulated silver wire was used as the indifferent electrode. The cardiac action potential was recorded by amplifying the potential difference between the electrodes with a high-input impedance amplifier and displaying the output on a DM 63 Tektronic storage oscilloscope using indirect calibration. The signals together with the endocardial and epicardial electrograms were recorded on magnetic tape for subsequent analysis. Selected periods were recorded on paper at 100 mm/s. Sequential measurements of action potential duration (95% repolarisation) were performed. Small variations in diastolic potential which might affect action potential duration determination were eliminated by time measurement from 95 per cent depolarisation to 95 per cent repolarisation. Endocardial-epicardial conduction time, defined as the time from onset of the R wave on the endocardial electrogram to the onset of epicardial activation on the intracellular recording, was also recorded sequentially. Epicardial activation was taken to be at the onset of phase O of the action potential. Small 'extrinsic' potentials resulting from conduction delay between intra- and extracellular electrodes and creating a small foot at the base of phase O were minimised by closer positioning of the reference electrode to the recording electrode and excluded from analysis. Closely reproducible data were obtained from multiple impalements at one site and from continuously recorded control measurements from one electrode before ischaemia. Absolute measurements of transmembrane potential and initial upstroke velocity were not considered valid on account of unavoidable signal distortion caused by movement artefact and variations in microelectrode tip potential. Satisfactory recordings could be maintained over the 5-minute period of occlusion. Data were disregarded after microelectrode tip breakage. Changes in mean epicardial ST segment elevation were derived by continuous computer analysis of the recorded extracellular electrograms (Luxton *et al.*, 1977).

Procedure

In 8 dogs, successive 5-minute occlusions of the coronary artery were achieved with a light spring releasable clip. A 30-minute period of recovery was allowed after each period of ischaemia. When ventricular fibrillation occurred, the occlusive clip was immediately removed and the heart defibrillated by DC countershock (20 watt-second). In no case was correction of acid-base balance required.

Recordings were made before and at 0·5-minute intervals after coronary occlusion of mean epicardial ST segment elevation, endocardial-epicardial con-

duction time, and cardiac action potential duration at 95 per cent repolarisation.

The effect of variations in heart rate was studied in a further 5 dogs again with a series of 5-minute coronary artery occlusions and 30-minute recovery periods. The first occlusion was undertaken at the dogs' intrinsic heart rate and served to condition the heart to the possible deleterious effects of ischaemia and reperfusion (see Discussion). The second occlusion was undertaken at a heart rate of 100 beats/minute achieved by combined vagal slowing and atrial pacing. During the third and subsequent occlusions, vagal stimulation was continued at the same rate, but the heart rate was increased in increments of 20 beats/minute to a maximum of 200 beats/minute for the seventh and final occlusion.

STATISTICS

All results are expressed as mean $\pm$ standard error of mean unless stated otherwise. The significance of difference was tested by Student's t test, paired or unpaired as appropriate.

Results

Before coronary artery occlusion, and with a heart rate of 175 beats/min maintained by atrial pacing, the mean action potential duration was 182 $\pm$ 9 ms in 8 dogs. Endocardial-epicardial conduction time was 24 $\pm$ 1 ms.

INITIAL CORONARY ARTERY OCCLUSION

Acute coronary artery occlusion was rapidly followed by changes in both the duration and shape of the action potential and increased endocardial-epicardial conduction time.

A representative example is shown in Fig. 1. Rapid action potential shortening was associated with an increase in endocardial-epicardial conduction time within 3 minutes of ischaemia. Potentials then assumed the appearance of 'slow response' potentials (Cranefield et al., 1972), with loss of the initial rapid upstroke phase. Electrical alternans of both action potential and ST segment elevation was evident by 3½ minutes of ischaemia, and 2:1 conduction block was recorded intracellularly after 4 minutes of ischaemia (Fig. 1). In some occlusions more irregular patterns of conduction block occurred.

Mean values over the period of the initial occlusion in 8 dogs are shown in Fig. 2. Mean action potential duration shortened to 140 $\pm$ 15 ms at 1 minute (P < 0·001) and 113 $\pm$ 9 ms at 2 minutes (P < 0·001) though transient prolongation of mean action potential duration was seen in 1 dog within 30 seconds. Loss of action potential amplitude and initial upstroke velocity was observed but not quantified (see Methods). The appearance of 'slow response' wave forms occurred earlier in animals where mean action potential duration shortening was rapid. The progressive shortening of mean
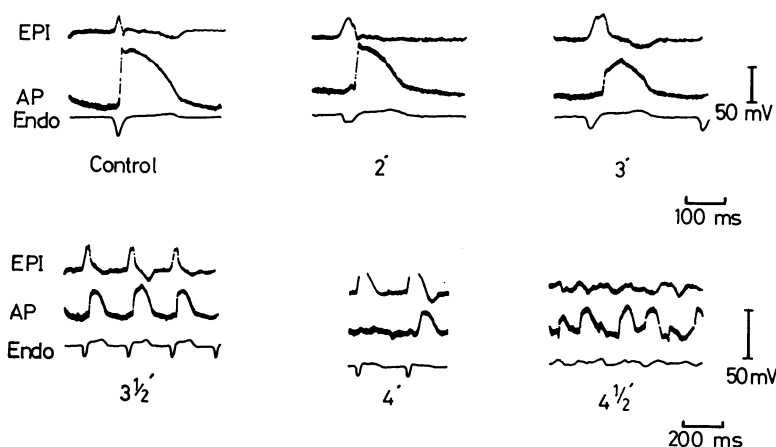

Fig. 1 Recordings of action potential (AP), endocardial (ENDO), and averaged epicardial (EPI) electrograms from dog heart in situ before and 2, 3, 3½, 4, and 4½ minutes after a coronary artery occlusion. Note shortening of AP at 2 minutes and progressive delay in its onset with respect to the endocardial potential. Electrical alternans occurs at 3½ minutes and localised 2:1 conduction block at 4 minutes recorded from intracellular but not extracellular electrodes. Ventricular fibrillation occurred at 4½ minutes.
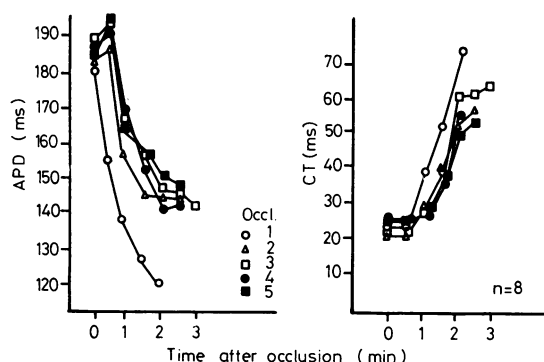
Fig. 2   *Effect of successive 5-minute coronary occlusions on action potential duration and conduction delay in 8 dogs. Note the more pronounced effect of the first occlusion compared with subsequent occlusions. Mean values are shown but data discontinued on initiation of VF in any dog.*

action potential duration was associated in every case with prolongation of endocardial-epicardial conduction time mean values, being 38 ± 5 ms at 1 minute and 71 ± 17 ms at 2 minutes (P < 0·05). These data are illustrated in Fig. 2 and listed in Table 1.

Interpretation of epicardial ST segment change was made difficult by the appearance of widened epicardial QRS complexes in 5 dogs with severe lengthening of conduction time. However, in 3

dogs, where lesser degrees of conduction time were not associated with QRS widening, mean ST increased to 3·5, 5·3, 7·7, and 11·1 mV at 1, 2, 3, and 4 minutes, respectively, after coronary artery occlusion.

SUCCESSIVE CORONARY ARTERY OCCLUSIONS

After the second coronary artery occlusion, mean action potential duration shortened and endocardial-epicardial conduction time lengthened but at a slower rate than after the first occlusion. Thus, mean action potential duration shortened from 185 ± 9 ms to 157 ± 12 ms at 1 minute, a length significantly greater than that at 1 minute after occlusion 1 (P < 0·005). Progressive prolongation of endocardial-epicardial conduction time was also more gradual than after occlusion 1 (Table 2). There was, however, no significant difference in the rate of changes in mean action potential duration and conduction time between occlusions 2, 3, 4, and 5 (Fig. 2 and Table 2). Control mean action potential duration showed slight successive prolongation before successive occlusions despite constant heart rate (Fig. 2).

The slower development of change in mean action potential duration and endocardial-epicardial conduction time was also associated with slower development of ST segment elevation in second and subsequent occlusions in those occlusions not developing gross intraventricular conduction abnor-

Table 1   *Effect of initial coronary occlusion on action potential duration and endo-epicardial conduction time*

Case no.	Preocclusion	Minutes after coronary occlusion				
	0	1	2	3	4	5
Action potential duration						
1	220	220	150	140	100	100
2	150	130	105	150	160	120
3	190	110	100	*		
4	180	140	150	150	170	155
5	190	130	80/140†	*		
6	190	150	120	115/160†	90/160†	*
7	155	98	85/120†	*		
8	185	175	120	120/140†	*	
Mean ± SEM	182 ± 8	140 ± 13	113 ± 9			
Endocardial-epicardial conduction time						
1	28	32	48	90	80	25
2	20	28	42	60	110	80
3	27	27	62	*		
4	20	20	24	30	32	33
5	24	44	100	*		
6	20	28	160	165	168	*
7	28	58	75	*		
8	26	28	52	68	*	
Mean ± SEM	24 ± 1	38 ± 5	71 ± 17			

*Onset of ventricular fibrillation.
†In these cases, alternans of the action potential occurred. The upper and lower limits are shown.

Table 2 *Effect of successive coronary occlusions on action potential duration, endocardial-epicardial conduction time, and incidence of ventricular fibrillation*

Occlusion no.	Preocclusion	Minutes after coronary occlusion				
	0	1	2	3	4	5
Action potential duration						
1	182 ± 8	140 ± 13	113 ± 9			
2	185 ± 8	157 ± 12	143 ± 9			
3	191 ± 8	170 ± 8	147 ± 6	136 ± 7		
4	190 ± 9	169 ± 8	140 ± 5			
5	188 ± 12	177 ± 13	153 ± 9			
Endocardial-epicardial conduction time						
1	24 ± 1	38 ± 5	71 ± 17			
2	24 ± 2	29 ± 4	51 ± 6			
3	25 ± 1	27 ± 1	49 ± 5	68 ± 5		
4	25 ± 1	28 ± 1	51 ± 6			
5	24 ± 2	28 ± 3	44 ± 9			
No. of animals with ventricular fibrillation						
1	—	—	1	3	4	5
2	—	—	—	2	2	3
3	—	—	—	—	3	4
4	—	—	—	1	2	4
5	—	—	—	1	3	4

Mean ± standard error of mean values from 8 dogs are shown.

mality. A representative example of change in mean ST segment elevation is produced in Fig. 3.

ONSET OF VENTRICULAR FIBRILLATION
Ventricular fibrillation followed in 5 of 8 dogs at a mean time of 3 minutes 23 seconds after the first occlusion and 4 minutes 44 seconds after the second occlusion. It developed in only 4 dogs after 3rd and
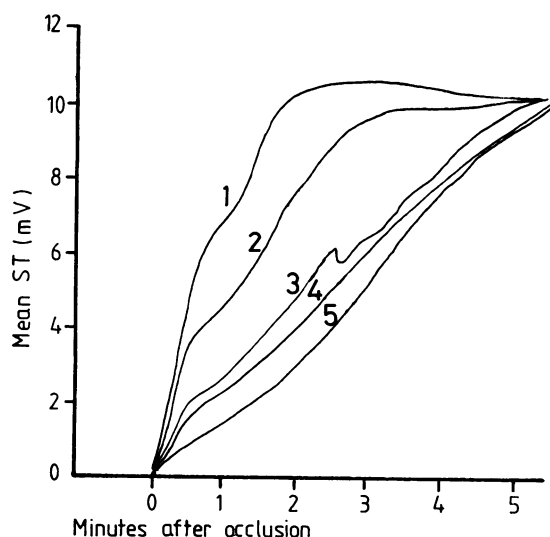

Fig. 3 *Effect of successive high coronary arterial occlusions on mean epicardial ST segment elevation in one dog. Data processed by an ST segment computer.*
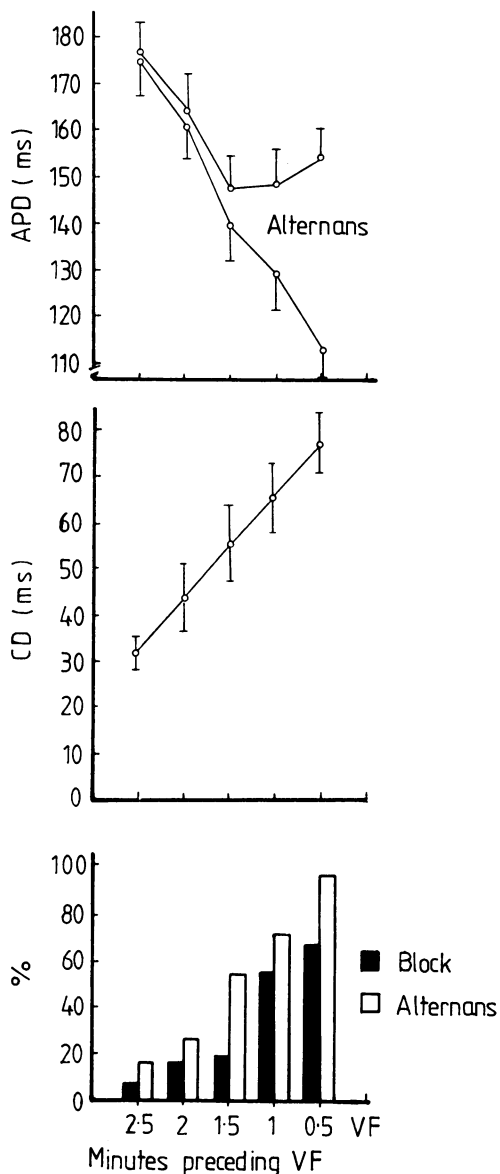
4th occlusions (mean times of onset 4 minutes 6 seconds, 3 minutes 33 seconds). In each case, this arrhythmia was preceded by the development of action potential alternans—both duration and amplitude. These changes were accompanied by epicardial and by endocardial ST segment alternans (Fig. 1), and by varying degrees of conduction blocks with either 2:1, 3:1, or more irregular patterns of endocardial-epicardial conduction block.

The temporal relations between changes in mean action potential duration, endocardial-epicardial conduction time, and electrical alternans or conduction block before development of ventricular fibrillation are shown in Fig. 4. Data are taken from 20 occlusions in 5 dogs all resulting in ventricular fibrillation. Ventricular fibrillation was never seen without shortening of mean action potential duration and lengthening of endocardial-epicardial conduction time. Electrical alternans was seen in 19 instances (95%) and conduction block in 13 (65%).

By contrast during occlusions not resulting in ventricular fibrillation, electrical alternans was observed in 72 per cent and conduction block in 33 per cent of instances during the 5-minute occlusion period. The mean time of onset of alternans was 3 minutes 12 seconds and block 4 minutes 8 seconds compared with 2 minutes 24 seconds and 2 minutes 48 seconds, respectively when ventricular fibrillation developed.

EFFECT OF HEART RATE
Controlled heart rates from 100 to 200 beats/minute were achieved with combined atrial pacing and

Fig. 4 *Changes in action potential duration, conduction delay, and frequency of AP alternans and block before onset of ventricular fibrillation. The two values of APD refer to alternating values.*

Fig. 5 *Effect of increasing heart rate in second and subsequent coronary occlusions in 5 dogs on APD and CT. Note the initiation of VF at higher rates.*

vagal stimulation, during occlusions 2 to 7 in 5 dogs. In every case control mean action potential duration was physiologically dependent on the heart rate, that is mean action potential duration before occlusion was shorter at faster heart rates. After coronary occlusion at slower heart rates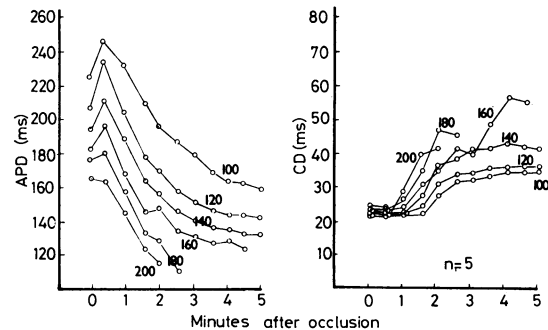, transient lengthening of the action potential duration was seen in every case. This was maximal at a heart rate of 100 beats/min where, after 30 seconds, mean action potential duration increased from 224 ± 35 to 245 ± 41 ms. After 1 minute, mean action potential duration shortened and endocardial-epicardial conduction time lengthened, as in the constant heart rate study, but the rate of progression became faster as the heart rate was increased with later occlusions. This was in contrast to the controlled rate study where changes tended to become milder with successive occlusions.

Mean ST segment elevation similarly showed progressively larger values with increase in heart rate. After 2 minutes of occlusion mean ST was 1·5, 2·1, 2·6, 4·8, 6·2, and 6·0 at 100, 120, 140, 160, 180, and 200 beats/minute, respectively.

Ventricular fibrillation was not seen at heart rates of 100 to 140 beats/min but occurred in 1 dog at 160 beats/min, 2 dogs at 180 beats/min, and 3 dogs at 200 beats/min.

These data are illustrated in Fig. 5.

Discussion

These studies show quantitatively the rapid electro-physiological changes occurring at myocardial cellular level, which precede the development of early ventricular fibrillation during acute myocardial ischaemia and amplify findings briefly described in a preliminary communication from our laboratory (Russell *et al.*, 1977). These changes are characterised by shortening of the action potential, prolongation of endocardial-epicardial conduction delay, and the appearance of potentials of 'slow response' type morphology. In addition, they are shown to be less severe during second and subsequent periods of ischaemia and at slower heart rates.

Direct recording of transmembrane action potentials in the beating heart was performed using

floating microelectrodes. We have modified previously reported techniques (Woodbury and Brady, 1956; Prinzmetal *et al.*, 1962; Czarnecka *et al.*, 1973) to permit more stable recordings from ischaemic myocardium in vivo. Restriction of cardiac movement by a metal ring to protect electrode impalement was not required since the fine distal tip of the electrode was supported proximally by a wedge of thicker glass. The problem of myocardial splinting causing alterations of regional blood flow was thus avoided. Measurements of absolute membrane transmembrane potential and upstroke velocity were not performed in our experimental preparation because of tip movement artefact and intermittent incomplete cellular impalement.

The effect of anoxia and ischaemia in shortening the action potential and decreasing its amplitude is well known (Trautwein *et al.*, 1954; McDonald and McLeod, 1973). These changes were clearly shown in the present study but occurred more rapidly than in previously reported in vitro studies of anoxic myocardium or isolated perfused pig hearts (Downar *et al.*, 1977b) and were particularly prominent after coronary occlusions that eventually resulted in ventricular fibrillation. For example, action potential duration after 3 minutes of ischaemia was 50 to 60 per cent of control and comparable to the degree of shortening achieved after 30 minutes of anoxia in isolated ventricular muscle preparations. This action potential shortening during ischaemia is thought to result from diminished energy-dependent calcium slow-flux under conditions of reduced ATP availability of adenosine triphosphate and to reflect the abnormal energy balance within ischaemic tissue (McDonald and McLeod, 1973). At slower heart rates, and therefore presumably with milder ischaemia, transient prolongation of the action potential was often noted during the initial 30 seconds after coronary occlusion. This effect after acute ischaemia could be related to the greatly prolonged potentials shown in chronically ischaemic conduction tissue (Friedman *et al.*, 1975).

The induction of 'slow response' type morphology potentials within 3 minutes of ischaemia presumably results from inactivation of rapid inward sodium current after rapid loss of membrane potential (Weidmann, 1955). Membrane depolarisation during ischaemia has been described in the dog (Samson and Scher, 1960; Prinzmetal *et al.*, 1962) and in the pig heart (Kléber *et al.*, 1978) and related to TQ segment depression of the extracellular electrogram. A loss of action potential amplitude was observed in our series though not quantified, which is consistent with these observations. This contrasts with findings during in vitro studies of anoxic myocardium that have demonstrated gross shortening of action poten-

tial but with maintenance of transmembrane resting potential. Slowing of intramyocardial conduction particularly between endocardium and epicardium during ischaemia is well described (Durrer *et al.*, 1971, Cox *et al.*, 1973) and has also been ascribed to the development of 'slow potentials' (Cranfield *et al.*, 1972). In the present study, action potentials with a morphology similar to these slow response potentials were associated with pronounced conduction delays of 50 to 150 ms.

Alternans both of amplitude and of duration of the action potential was observed between 2 and 5 minutes of ischaemia and was most obvious during slow response activity. The alternans increased in magnitude with time and in several dogs developed into intermittent conduction block with either a 2:1 or more irregular patterns. These changes were reflected in the surface electrocardiogram as ST segment alternans. Similar alternating phenomena have been described in ischaemia (Downar *et al.*, 1977b) and in association with arrhythmias (El-Sherif *et al.*, 1977). In our series electrical alternans of the action potential preceded ventricular fibrillation in 95 per cent of cases and epicardial conduction block preceded fibrillation in 65 per cent. Moreover, both alternans and conduction block occurred earlier and more frequently in occlusions resulting in fibrillation. Electrical alternans could arise either by a true cellular potential alternans (Kleinfeld and Stein, 1968) or by means of electrotonic interaction between adjacent areas of intermittent conduction block (Hellerstein and Liebow, 1950). An increase in diastolic excitability threshold, as may follow an increase in extracellular potassium, could account for the conduction blocks observed in this study (Elharrar *et al.*, 1977). Alternatively, a myocardial depressant factor has been isolated from ischaemic venous effluent blood with pronounced depressant effects on excitability (Downar *et al.*, 1977a). Electrical uncoupling of hypoxic or anoxic cardiac cells has been shown in vitro and may contribute to inhomogeneity of impaired ventricular conduction during ischaemia (Wojtczak, 1976). Though the association between the electrical phenomena and fibrillation is most striking it need not necessarily imply a cause and effect relation.

The presence of 'slow response' activity and distinct endocardial-epicardial conduction delays however would provide the exact setting for initiation of arrhythmias by re-entrant excitation. In addition, local heterogeneity of unidirectional conduction block may result from regional variations in diastolic excitability threshold. It is possible, therefore, that quite local discrete regional asynchrony of excitability occurring simultaneously in many regions of the ischaemic ventricle may be of impor-

tance in the initiation of ventricular fibrillation. Multifocal re-entrant excitation would then be possible in areas of myocardium of a few millimetres diameter. Abnormal automaticity which is an alternative possible mechanism has been shown in 'slow response' ventricular muscle fibres as well as in conducting tissue (Imanishi and Surawicz, 1974). No such activity however was recorded during any cellular impalement preceding ventricular fibrillation arguing against its role in early ventricular arrhythmogenesis.

Observations during later occlusions at varying heart rates confirmed the findings of other workers (Han, 1969; Kent *et al.*, 1973) that the vulnerability of the heart to ventricular ischaemia is enhanced with increasing heart rate. In the present study, ventricular fibrillation did not occur at rates below 140 beats/min. At higher heart rates the relation between earlier onset of fibrillation and more rapid changes in action potential and conduction during ischaemia is consistent with re-entrant excitation and strongly supports a re-entrant mechanism for the early phase of cardiac arrhythmias after acute myocardial ischaemia.

Another result of these studies is one of practical importance for those investigating experimental myocardial infarction in that electrophysiological measurements have been shown to be different during the first compared with subsequent occlusions. Thus, in the 5 dogs which developed ventricular fibrillation, the mean time of onset of this arrhythmia occurred 45 or more seconds earlier during the first occlusion. Similarly, after later occlusions, a slower rate of shortening of action potential and prolongation of conduction delay, together with less ST segment elevation, was observed despite comparable heart rates. These findings suggest enhanced vulnerability of the myocardium to arrhythmias during initial coronary artery occlusions and are consistent with an altered ischaemic response in the relevant area of myocardium during successive periods of occlusion. This phenomenon is compatible with the finding of diminished contractility in myocardium reperfused after transient ischaemia (5 to 10 minutes coronary occlusion) (Vatner *et al.*, 1975; Smith *et al.*, 1978). If ischaemia damages the contractile apparatus, a situation of reduced oxygen demand, and hence less oxygen debt, could then be created during a second or subsequent occlusion. Milder changes during a second or subsequent occlusion may reflect some adaptive mechanism controlling coronary blood flow and related to greater vasodilator activity consequent upon release of vasoactive adenine nucleotides (Berne and Rubio, 1969). Alternatively, depletion of noradrenaline during the first occlusion could alter the metabolic and electrophysiological responses to successive occlusions. This lack of reproducibility of electrophysiological data between the initial and subsequent periods of ischaemia indicates that studies by other workers employing similar experimental models of repetitive coronary occlusion should be critically reappraised. Comparative studies of metabolic or pharmacological intervention during ischaemia may have little validity if not performed after an initial sham occlusion.

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